REMARKS

Summary of the Office Action

Claims 1-46 are pending. Claims 1-23 and 25-46 are rejected. The Examiner also objected to claim 24.

Support for Claim Amendments

Support for the amendments to claims 1 and 2 is found, for example, on page 10, lines 28-30.

Support for the amendment to claims 9 and 10 is found, for example, on page 7, line 33 to page 8, line 3.

Support for the amendment to claim 17 is found, for example, on page 2, lines 10-13 and lines 21-24.

Support for the amendment to claim 25 is found, for example, on page 15, lines 17-20.

Support for the amendment to claims 20, 30 and 38 is found, for example on page 15, lines 2-24.

Support for new claim 48 is found, for example, on page 24, line 32 to page 25 line 2.

Support for new claim 49 is found, for example, on page 7, line 31 to page 8 line 3.

Support for new claim 50 is found, for example, on page 6, line 31 to page 7, line 12.

New claim 47 consists of claim 24 and its base claims rewritten in independent form.

Priority

The Examiner acknowledged Applicants' claim for domestic priority under 35 USC 119(e); however, the Examiner also alleged that the priority application fails to provide adequate support under 35 USC 112 for claims 4-9, 33-40 and 43-46. The Examiner alleged that the provisional application does not provide adequate written description for the indicated claims, and he used the filing date of the non-provisional application to determine the patentability of the above claims over the prior art.

The Applicants submit that adequate written description for the claims was present in the provisional application. Claims 4-9, 33-40 and 43-46 define an invention

that was clearly conveyed to those skilled in the art at the time the provisional application was filed.

The *Guidelines for the Examination of Patent Applications under the 35 USC 112, paragraph 1, Written Description Requirement* (the "Written Description Guildelines") include the following principles (see 66 FR 1099 (Jan 5, 2001 at) p. 1105). There is a *strong presumption* that an adequate written description of the claimed invention is present when the application is filed. The subject matter of the claim need not be described literally (i.e., using the same terms or in haec verba) in order for the disclosure to satisfy the description requirement. Claims may be supported in the specification through express, implicit or inherent disclosure. The fundamental factual inquiry is whether the specification conveys with reasonable clarity to those skilled in the art that, as of the filing date, applicant was in possession of the invention as now claimed.

Claims 4-7 include methods of differentiating pluripotent ES cells at particular cell densities, such as, greater than 0 cells/μl to 50 cells/μl; greater than 0 cells/μl to 20 cells/μl; greater than 0 cells/μl to 10 cells/μl; and 10 cells/μl.

The provisional application clearly describes the culturing of ES cells at low density and specific examples of such low densities are provided. Under the heading of "Culture Media," it is stated on page 9, lines 25-29 that, "The culturing of the cells at low density in the serum-free media, includes the understanding that the conditions under which the cells are cultured are appropriate for the continued survival of the cells for the purposes for which the cells are being used." It is also stated on page 12, lines 16-17 that "Low cell density preferably refers to cell culture densities of between 1 cell/μl and 50 cells/μl." Page 4, lines 12-18 refers to "culturing the ES cells at low density in the serum-free media, preferably the ES cells are cultured at a density between about 1 cell/μl and about 50 cells/μl, more preferably the ES cells are cultured at a density of 20 cell/μl."

Claim 8 refers to methods involving no EB formation. This is adequately described by page 3 at lines 24-28 which refer to, "In accordance with the present invention, it has surprisingly been discovered that in low-density cell culture assays, in the absence of serum-derived or feeder cell-derived factors and in the absence of embryoid body (EB) formation, ES cells directly differentiate into neural cells."

Amended claim 9 states that the differentiating ES cells form at least one sphere colony. This is described by Figure 1 and its legend in the provisional application. Page 13, line 22 to page 15, line 2 describes a limiting dilution analysis in which cell numbers were adjusted to give a starting concentration of 5000 cells/ml from which serial dilutions were made. Final cell dilutions ranged from 1000 cells per well to 1 cell per well in 0.5 ml aliquots. The Applicants also provided a method to calculate the number of cells required to form one sphere colony.

Claims 33-40 include methods for screening for modulators of cellular differentiation, methods for screening for differentiation factors of cellular development and modulators obtained by these methods. These claims are supported by, for example, page 4, line 19 to page 6, line 14, which disclose methods of differentiating primitive neural stem cells in the presence of a cytokine and growth factor. Figure 1A and its legend disclose neural sphere colony formation in the presence of various cytokines and growth factors and combinations thereof.

Claims 43-46 include methods for treating neurodegenerative disorders, methods for the treatment of any disease or conditions resulting from cell loss or function in the neural system and methods of gene therapy. Page 3, lines 14-18 discusses the usefulness of ES systems and neural cell systems for the development and testing of drugs for the treatment of developmental and cerebral neural anomalies and neuropathies. Fig. 6 of the application shows that the primitive neural stem cells can be transplanted into the earliest stages of an embryo and differentiate into other cell types, which provides additional support for the transplantation and treatment claims.

Specification

The Examiner objected to typographical errors in the disclosure. The Applicants corrected the cited typographical errors.

Claim Objections

The Examiner objected to typographical errors in claims 9 and 46. The typographical errors have been corrected. The term term "neurosphere" in claims 9 and 10 has been replaced with "sphere colony".

The multiple dependency in claim 14 has been corrected.

Claim Rejections - 35 U.S.C. § 101

Claims 25-27 and 39 were rejected under 35 USC 101 on the basis that the claims are directed to non-statutory subject matter. As suggested by the Examiner, the claims have been amended to include the term "isolated" in reference to cells, modulators and differentiation factors.

Claim Rejections - 35 U.S.C. § 112, first paragraph

Claims 17, 31, 33-35, 37, 38, 40 and 41 were rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the invention.

The Examiner alleged that claim 17 is not supported by adequate written description in relation to inhibitors of TGF-β related signaling. Amended claim 17 refers to "TGF-β superfamily signal transduction." The Examiner stated that the application does not provide adequate written description for the broad class of any and all inhibitors of TGF-β related signaling. The Examiner further states that in an unpredictable art, adequate written description cannot be achieved by disclosing only one species within the genus. The Examiner suggested that the written description requirement may be satisfied through sufficient description of a representative number of species, by reduction to practice, reduction to drawings or by disclosure of relevant identifying characteristics. The Applicants submit that the application includes more written description than was identified by the Examiner. The Examiner noted that the application provides working examples using the BMP (bone morphogenetic protein) antagonists Noggin and Cerberus. On page 6 and in claim 19, the application refers to "the Cerberus family of proteins." This description is further supported by inclusion in the application of articles describing the family (Pearce, J.J.H., Penny, G., Rossant, J. (1999). A mouse cerberus/DAN-related gene family. Dev. Biol. *209*, 98-110; Belo, J.A., Bouwmeester, T., Leyns, T., Kertesz, L., Gallo, N., Follettie, M., De Robertis, E.M. (1997). Cerberus-like is a secreted factor with neuralizing activity expressed in the anterior primitive endoderm of

the mouse gastrula. Mech. Dev. 68, 45-57; Bouwmeester, T., Kim, S.H., Sasai, Y., Lu, B., De Robertis, E.M. (1996). Cerberus is a head inducing secreted factor expressed in the anterior endoderm of Spemann's organizer. Nature 382, 595-601). Thus the application describes a significant number of inhibitors of TGF-β related signaling and provides a representative number of species. It is noted in the Written Description Guidelines at page 1106 that description of a representative number of species does not require the description to be of such specificity that it would provide individual support for each species that the genus embraces. The disclosure in the application reasonably conveys to the artisan that the Applicants have possession of the claimed subject matter.

The Examiner has also stated that Noggin and Cerberus are structurally distinct molecules and that the Applicants have not provided the relevant identifying structural characteristics that would confer on a molecule the function of an inhibitor of TGF-β related signaling. There is no requirement to disclose common structural characteristics that confer on Noggin and Cerberus the function of an inhibitor of TGF-β related signaling. The Applicants may disclose any sufficient combination of relevant, identifying characteristics, such as structure or other physical and/or chemical properties. The Applicants submit that the class of compounds that are inhibitors of TGF-β related signaling are adequately defined in the art (see, for example, Pearce et al, above). Therefore, specific structural differences between Noggin and Cerberus do not need to be described in order to describe the claimed inhibitors. Such inhibitors are readily identified through articles and assays in the art. As well, as described below, any compound that is an inhibitor of TGF-β related signaling is useful according to the claimed invention, irrespective of its structure.

The Applicants respectfully disagree with the Examiner's assertion that the art is unpredictable and requires a higher level of written description. The art cannot be said to be unpredictable after this invention and a review of the teachings in the application. The Applicants' results clearly show that TGF-β signaling (shown by BMP4 data) has a specific effect in limiting the numbers of single ES cells that differentiate into colony forming neural stem cells and that inhibition of this pathway is sufficient to enhance primitive neural stem cell colony formation. To determine more directly the effect of blocking TGF-β (BMP) signaling, in Example 4, the Applicants used an ES cell line

with a targeted null mutation in the *Smad4* gene (an intracellular transducer of TGFβ-related signaling) to show the effect of blocking BMP signaling (i.e. inhibition of TGFβ-related signaling). The results provide a strong basis to assert the usefulness of any inhibitor of TGFβ related signaling, despite the existence of variant species of inhibitors. The knock out experiments clearly show that any compound that causes inhibition of TGFβ-related signaling will enhance embryonic stem (ES) differentiation toward primitive neural stem cells. This teaching, in combination with the knowledge in the art of TGF-β inhibitors, allows one of skill in the art to recognize that the Applicants were in possession of all the necessary common attributes possessed by the members of the genus in view of the species disclosed.

Claims 31, 33-35, 37, 38, 40 and 41 were rejected on the basis that they encompass a method of producing any and all cell types or methods of screening compounds that induce differentiation toward any and all cell types. The Examiner acknowledged that the claimed methods for producing or screening for differentiation toward neural cells meet the written description. The specification teaches the in vivo differentiation of primitive neural stem cell-derived cells into neural and non-neural cell types, and therefore the claimed invention is commensurate with its scope. The application describes that it is generally known that embryonic stem cells differentiate into neural or epidermal cells. Figure 6 shows that the primitive neural stem cells can form many types of cells, not just neuronal cells. When transplanted into an embryo, the primitive neural stem cells colonize many different tissues (fig. 6). The primitive neural stem cells are therefore capable of differentiating in to neuronal cells and non-neuronal cells. The application also states beginning on page 16, beginning at line 29, that the primitive neural cells of the invention are pluripotent and can be used in developing tissues, neural or otherwise. It is also stated on page 17, line 2 that, "Such tissues could be used in transplantation therapy for conditions other than those related to the neural system. For instance, the cells of the invention could be used to develop insulin producing cells for the treatment of diabetes. The cells could also be used to develop a desired cell type by propagating them under predetermined conditions conducive to development of such cell type. The conditions for ES cell differentiation into multiple cell types are also described in the application by reference to J. Yamashita et al, Nature

408 page 92." Thus, the application includes adequate written description in relation to neuronal and non-neuronal cells.

Claims 1-19, 28, 29 and 37-46 were rejected under 35 USC 112, first paragraph, for lack of enablement. The Examiner stated that the application does not provide enablement for differentiation in the presence of a feeder layer. The claims have been amended without prejudice to recite that the methods are practiced in "in serum-free and feeder-layer free media."

Claims 20-23 were rejected under 35 USC 112, first paragraph, on the basis that the application does not reasonably provide enablement for methods wherein cells are cultured in the absence of LIF or B27. The Applicants have amended claim 20 to recite a "growth factor or survival factor." The application states that LIF acts as a "survival factor (reviewed in Mehler and Kessler, 1997) that is initially required for ES cell viability" (page 47). Examples of other suitable growth factors and cytokines are provided, for example, on page 12, line 26 to page 13, line 14. The application also sets out a clear and reliable assay to determine whether secondary primitive neural stem cell colonies are formed. No undue experimentation would be required.

Claims 44-46 were rejected under 35 USC 112, first paragraph, as lacking enablement. Applicants respectfully submit that a skilled artisan, once put in possession of the primitive neural stem cells of the present invention, would rely on the teachings in the specification and the general knowledge in the art with respect to transplantation of cells. The application shows how to use the primitive neural stem cells for transplantation to treat neurodegenerative disorders and diseases or conditions resulting from cell loss or function in the neural system. The Applicants have provided chimera data in fig. 6 of the application to show that the primitive neural stem cells can be transplanted into the earliest stages of an embryo and differentiate into other cell types. Therefore, transplanting primitive neural stem cells would be effective in other tissues. The Applicants' chimera data also shows that the primitive neural stem cells do not affect normal development. There is no deleterious effects to the tissue and viability of the host. The primitive neural stem cells are the best candidate cell type to transplant because these cells are not an aggregation of many different neural and non-neural cell types that are already committed to specific lineages (as in the case of embryoid bodies)

and there are no elaborate culturing procedures that are required to get specific cell types (like glial precursors from ES cells). The primitive neural stem cells will respond to the appropriate environmental cues after transplant and differentiate accordingly.

Many other research groups have been transplanting cells into the central nervous system to obtain neural specific differentiation and/or functional recovery. These groups have already transplanted ES cell derivatives (glial precursor or embryoid bodies) without deleterious effects. Some examples are provided below (copies of articles enclosed).

1. Brustle et al. (1999) Science 285(5428): 754-756

ES cells were differentiated to glial cells and then transplanted to attempt to recover function in an MS disease rat model. The ES cell-derived precursors interactd with host neurons and efficiently myelinated axons in brain and spinal cord. The authors state on page 75 that their data shows that, "cell type-specific somatic precursors can be generated from ES cells and used for nervous system repair."

2. McDonald et al (1999) Nat. Med. 5(12): 1410-1412

Cell transplants into an injured spinal cord model produced axonal growth or functional benefits. On page 1411 it was stated that "mouse ES cell-derived cells, when transplanted into the spinal cord 9 days after weight-drop injury, survive for at least 5 weeks; migrate at least 8mm away from the site of transplantation; differentiate into astrocytes, oligodendrocytes and neurons without forming tumors; and produce improved locomotor function."

3. Yang et al. (2002) Exp. Neurol. 177(1): 50-60

This reference is after the Applicants' filing date, but is relevant to show that the Applicants' invention is enabled and works as described in the application. A neural stem cell line derived from the embryonic cerebellum was transplanted into striatum, including Parkinsonian rat striatum, to determine patterns of differentiation into neural cell types in the brain. The cells expressed and underwent specific expression of dopaminergic traits (markers associated with neuronal differentiation). By 2-5 weeks post-grafting, in the

majority of these transplants, nearly all engrafted cells expressed the dopamine-synthesizing enzymes tyrosine hydroxylase and aromatic L-amino decarboxylase.

4. Berringer et al (2000) Brain Pathol. 10(3): 330-341

ES cells were formed into embryoid bodies and then transplanted into different brain regions to obtain appropriate differentiation into neural cells.

Claims 17-19 were rejected under 35 USC 112, second paragraph, as indefinite. In particular, the Examiner objected to the phrase "inhibitor of TGF-β related signaling." The Applicants have amended their claims to recite "inhibitors of TGF-β superfamily signal transduction." The Applicants used BMP inhibition in their examples, and BMP is a member of the TGFβ superfamily. The application also identifies other members of the family, such as activin, on page 2, lines 10-13. The amended claim clearly defines the relationship of the signaling pathways to TGFβ.

The Examiner also stated that claim 19 is indefinite because it is drawn to the "Cerberus" family of proteins. As noted above, this family is known in the art as exemplified by the Pearce et al. article. "Cerberus family" is clear and well defined to those skilled in the art.

Claim 36 was rejected as indefinite for referring to "modulators." The claim has been amended without prejudice to delete "modulators."

Claim Rejections - 35 U.S.C. § 102

Claims 4 and 5 were rejected as anticipated by Tropepe et al. (April 2001) *Neuron* 30:65-78. As noted above, the Applicants respectfully disagree that the priority date for these claims is September 28, 2001. However, solely in the interest of expediting prosecution, the inventors enclose a declaration of Drs. van der Kooy and Tropepe relating to this article. The Declaration establishes that they are the inventors of this work and that the co-authors of the cited art carried out experimental work under the control and direction of Drs. van der Kooy and Tropepe and did not contribute to the claimed concepts.

Claims 25, 26, 29-31 and 39 were rejected under 35 USC 102(b) as anticipated by Friachard et al (1995) *J. Cell Sci.* 108:3181. Claim 25 has been amended to refer to

"isolated primitive neural stem" cells. Even though Fraichard et al. demonstrate nestin expression and multipotentiality, there is no teaching that the cells are stem cells. Fraichard et al. discloses that ES cells can differentiate into neural precursor cells first (nestin-positive) and then that these precursor cells differentiate further into astrocytes, oligodendrocytes and functional neurons. The Examiner infers that the ES derived neural precursors are multipotent. However, Fraichard et al. does not have experiments to prove that a single neural precursor cell gave rise to many neurons and glia (i.e. establishing clonality). It is possible that "separate" neuronal and glial precursors differentiated from the ES cells en masse. In this scenario, these cells would still be nestin-positive and then differentiate into mature neurons and glia, but they would not have originated clonally from a stem cell. The Examiner implies that the neurons and glia came from multipotent precursors, but again this was not shown. More importantly, there were no explicit experiments to show that the neural precursor cells can self renew (i.e. had stem cell properties). Self-renewal is a key characteristic of stem cells. There is also no reference to the words "neural stem cell" in Fraichard et al. Since Fraichard et al. did not explicitly test whether the original multipotent precursor could repeatedly give rise to neurons and glia when passaged in culture, it cannot be asserted as an enabling disclosure of stem cells. The Fraichard et al. starting cell population is different than the isolated population of primitive neural stem cells that are used in this application.

The Examiner states on page 11 of the Office Action that there is "no evidence" in the specification to support claim 29 that the cells of the invention have a greater degree of pluripotential fates than do the definitive neural stem cells obtained by other means. However, the Applicants do provide evidence of pluripotentiality in Figure 6 and its legend in the description of the chimera experiments which show that ES derived neural stem cell colonies are competent to colonize many different tissues. The cells of the invention are thus capable of differentiating into neuronal cells and non-neuronal cells.

The Examiner alleges that it is not possible to argue that the primitive neural stem cells in Fraichard et al. are different from the cells of the invention without a direct side by side comparison because the authors do not present an exhaustive analysis of all potential fates for the described cells. The Applicants strongly disagree with the

Examiner's assertion that Fraichard discloses primitive neural stem cells. Fraichard did not isolate a primitive neural stem cell because retinoic acid was used in culture media. Retinoic acid causes the ES cells to become definitive neural stem cells or multipotent neural precursor (progenitor) cells. The inventors show that in the absence of retinoic acid, serum and a feeder layer, there is a stage where ES cells form a primitive neural stem cell. There are clear differences between primitive neural stem cells and other cell types, such as definitive neural stem cells and multipotent neural precursor cells. First, primitive neural stem cells need a compound such as LIF to form a sphere colony colony. Definitive neural stem cells and multipotent neural precursor cells do not need LIF to form a colony. Second, the primitive neural stem cells are pluripotent as discussed above. When transplanted into an embryo, many types of neural and non-neural cells are produced (figure 6). Definitive neural stem cells and multipotent neural precursor cells make only neural cells, not other types. Such cells would colonize tissues at a much lower frequency than primitive neural stem cells. Third, the gene expression profile for primitive neural stem cells is unique. The application provides data comparing definitive neural stem cell sphere controls with primitive neural stem cells (RT-PCR primitive neural stem cells derived from ES; RT-PCR embryonic brain). Figure 2 shows RT-PCR analysis of neural and non-neural lineage gene expression in RNA extracted from primary ES cells (R1), ES cell derived sphere colonies (SC), and positive control tissue samples (+) Figure 2B shows RT-PCR analysis of neural and non-neural lineage gene expression in RNA extracted from primary ES cells (R1), ES cell derived sphere colonies (SC), and positive control tissue samples (+). This gene expression profile allows the primitive neural stem cells to be distinguished from other types of cells. Thus, the primitive neural stem cells and the product claim are novel and inventive compared to Fraichard et al.

The Examiner also objected to claims 30 and 31 in view of Fraichard. The claims are directed to a primitive neural stem cell, which, for the reasons described above, patentably distinguishes the claims from Fraichard et al.

Claim 39 was rejected as allegedly reading on Fraichard. Claim 39 has been amended to recite "primitive neural stem cells" instead of pluripotent cells. The cells and

method in the amended claims are not the same as those taught by Fraichard et al. and do not include retinoic acid.

Claims 25, 26 and 29-32 were rejected under 35 USC 102(b) as anticipated by Okabe et al. (1996) *Mechanisms of Development* 59:89-102.

The amended claims recite primitive neural stem cells. Okabe et al. do not isolate or disclose a primitive neural stem cell, for reasons similar to those provided with respect to Fraichard et al. The Okabe et al. paper makes ES cells into embryoid bodies first before they culture neural precursors from the embryoid bodies. Okabe et al. do not have any experiments to show that a single neural precursor cell gave rise to many neurons and glia (i.e. establishing clonality). The Examiner implies that the neurons and glia came from multipotent precursors, but this was also not shown in Okabe et al. There were also no experiments to show that the neural precursor cells can self renew. Okabe et al. do not show whether the original multipotent precursor could repeatedly give rise to neurons and glia when passaged in culture and it cannot be asserted as an enabling disclosure of stem cells.

Claims 33-40 were rejected under 35 USC 10(b) as anticipated by Tropepe et al. (1999) *Soc. Neurosci Abstracts*. As noted above, the Applicants respectfully disagree that the priority date for these claims is September 28, 2001. Therefore, claims 33-40 would not be anticipated or obviated by Tropepe et al.

The Applicants acknowledge the Examiner's finding that claim 24 includes allowable subject matter. The claim has been rewritten in independent form as claim 47.

Based on the foregoing remarks, Applicants respectfully request that the rejection of the claims be withdrawn.

CONCLUSION

Applicants submit that the claims are in condition for allowance and such action is respectfully requested. If there are any charges or any credits, please apply them to Deposit Account No. 04-1406.

Respectfully submitted,

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Enclosure:
1. Brustle et al. (1999) Science 285(5428): 754-756
2. McDonald et al (1999) Nat. Med. 5(12): 1410-1412
3. Yang et al. (2002) Exp. Neurol. 177(1): 50-60
4. Berringer et al (2000) Brain Pathol. 10(3): 330-341
5. Declaration by Drs. van der Kooy and Tropepe

MARKED UP DRAFT OF AMENDMENT

In the Claims:

Please amend claims 1-3, 9, 10, 13, 14, 17, 19, 20, 25, 28-30, 33, 36, and 37 as follows:

1. (Amended) A method for differentiating one or more pluripotent embryonic stem (ES) cells [toward one or more neural cells] comprising:
(a) culturing the ES cells at low density in a [the] serum-free and feeder-layer free media; and
(b) allowing said ES cells to differentiate to [toward the] primitive neural stem cells.

2. (Amended) The method according [faccording] to claim 1 for differentiating embryonic stem cells to cells with markers characteristic of neural cells comprising:
(a) culturing the embryonic stem cells in the [a] serum free and feeder-layer free media at low cell density wherein said density is selected to minimize ES cell aggregation or EB formation; and
(b) allowing said cells to differentiate.

3. (Amended) The method of claim 2 wherein the cell density is selected as to avoid EB formation.

9. (Amended) The method of claim 7 wherein the differentiating ES cells form at least one sphere colony [neuro sphere].

10. (Amended) The method of claim 1 wherein the differentiating ES cells form at least one sphere colony [neurosphere].

13. (Amended) The method of claim 12 wherein the [ES cells differentiate into a] primitive neural stem cells are [cell, that is] pluripotent.

14. (Amended) The method of claims [claim] 1 or [and] 12 wherein the serum free media further comprises a growth factor.

17. (Amended) The method according to claim 1 wherein the media comprises an inhibitor of TGF-ß superfamily signal transduction [TGF-ß -related signaling].

19. (Amended) The method of claim 17 [18] wherein the inhibitor is selected from the Cerberus [Cerebus] family of proteins.

20. (Amended) A method for producing secondary primitive neural stem cell colonies comprising:

(a) culturing ES cells in low cell density [completely defined] serum-free and feeder-layer free media for a time and under conditions sufficient to differentiate the said ES cells to primary primitive neural stem cell colonies;
(b) dissociating and subcloning the primary primitive neural stem cell colonies generated from the said ES cells; and
(c) administering a growth factor or survival factor to the dissociated neural cells to produce secondary primitive neural stem cell colonies.

25. (Amended) An [One or more] isolated primitive neural stem cell [cell(s)] expressing one or more neural precursor cell marker [marker(s)] and/or one or more neural-specific mRNA molecule [molecule(s)], and having multilineage potential.

28. (Amended) A method according to any one [anyone] of claims 1 or 12 for analyzing the role of genes in the regulation of neural fate specification.

29. (Amended) An isolated [A] primitive neural stem cell produced by the method of claim [claims] 12 that comprises neural cell markers and is pluripotent.

30. (Amended) An isolated [A] primitive neural stem cell[produced comprising at least one neural cell marker and is pluripotent].

33. (Amended) A method for screening for modulators of primitive neural stem cell [cellular] differentiation comprising:
(a) culturing primitive neural stem [pluripotent] cells in serum-free and feeder-layer free media under low density conditions in the presence of the potential modulator;
[(b) allowing for differentiation of the cells] under conditions that produce differentiation in the absence of the modulator;
(b) [(c)] detecting any differentiation of the cells and cell types generated, if any; [.]
(c) determining whether the modulator affects the differentiation of the cells.

36. (Amended) A method of claim 35 for screening for [modulators or] differentiation factors of neural cell development.

37. (Amended) A method for screening for differentiation factors of cellular development comprising:
(a) culturing the cells of claim 29 in serum free media, in the presence [present] of the differentiation factor.
(b) detecting any differentiation of the cells.

Please renumber claims 38-45 to claims 39-46 and amend the same as follows:

39[38]. (Amended) An isolated [A] modulator or differentiation factor detected by the methods of claims 33-37.

40[39]. (Amended) A method according to claim 38 for modulating cellular differentiation.

41[40]. (Amended) The method of claim 1 for obtaining a homogenous uniform cell base.

42[41]. (Amended) The method of claim 40 wherein the cell base is a neural cell base.

43[42]. (Amended) A method for supplying cells for transplantation comprising culturing cells pursuant to the method of claim 1 or 12.

44[43]. (Amended) A method for treating neurodegenerative [neurdegenerative] disorders comprising administering to a patient in need thereof the cells of claim 29.

45[44]. (Amended) A method for the treatment of any disease or conditions resulting from cell loss or function in the neural system comprising [comprising] administering the cells of claim 29 to a patient in need thereof.

46[45]. (Amended) A method of gene therapy [thereapy], wherein the cell of claim 29 is modified to express a gene of interest and administering said [siad] modified cell to a patient in need thereof.

In the Specification:

Please replace the paragraphs from page 13, line 33 to page 15, line 20 with the following:

> The derivation of neural cells (among other cell types) from EB derived cells in vitro has been previously documented (Doetschman et al., 1985). Several studies have shown that the differentiation of neurons and glial precursors from EB derived cells can be enriched in the presence of retinoic acid (Bain et al., 1995; Fraichad et al., 1995; Strubing et al., 1995), FGF2 (Okabe et al., 1996), or PDGF (Brustle et al., 1999). Also, BMP4 has been shown to suppress neuronal differentiation of EB derived cells (Finley et al., 1999). Although these observations clearly demonstrate the potency of such factors to promote or attenuate neuronal differentiation of ES cells, each experiment was preceded by EB formation in the presence of serum. Here it is present an alternative and specific paradigm for neural cell fate specification directly from ES cells. Neural colonies can develop from ES cells in serum-free conditions in the

absence of EB formation, and many single ES cells can adopt a neural (nestin+) or neuronal (βIII-tubulin+) phenotype in the absence of exogenous growth factors. The derivation of neural cells from ES cells is preferrably carried out at relatively low cell densities in serum-free media. Low cell density as used herein refers to a cell culture density at which cell proliferation can occur with minimal and preferably no aggregation of ES cells or EB formation. Such densities are preferably about 50 or fewer cells/μl, most preferably less than 20 cells/μl, and even more preferred 10 or fewer cells/μl. It has been shown and a person skilled in the art would understand that the invention requires at least 1 cell to work, as such a cell density of greater than 0 is required. The present inventors have found (data not shown) that methods of the invention work significantly better in conditions where at about 10 or fewer cells/μl. Such a density results in a more homogenous cell culture i.e., primitive neural and neural cells as the case may be. The inventors have found that at this cell density, early mesodermal markers flk1 and brachyury are not expressed in the neurospheres derived clonally at lower densities from the novel single primitive neural stem cells of the invention. At higher densities, there is a greater likelihood that some, but not necessarily all [e] that form [from] by aggregation of ES cells that then differentiate to multiple tissue lineages and express the early mesodermal markers as noted above.

The experiments conducted by the inventors (data not shown) showed that single ES cells at such low densities will become neural stem cells. This is known because the single cells clonally proliferated to form spheres of 10,000 to 15,000 cells, all of which stained for an early ectodermal marker (nestin) and which do not express markers of other types of tissue like mesoderm, such as flk and brachyury. When a single sphere, clonally derived from a single primitive neural stem cell (the novel cell of the invention that comes from the neural differentiation of a single undifferentiated ES cell), is dissociated the small number of neural stem cells in the sphere (that come from the symmetrical division of the original

primitive neural stem cell) will proliferate to form secondary neurospheres (thus demonstrating self-renewal) that again all stain for the early neural marker nestin. In additional data (not shown), these new cells were determined not to be a tissue culture artifact but actually detectable in embryonic [fembryonic] day 6 and 7 epiblast in the mouse. These cells can be isolated by their ability to form neurospheres in the in the presence of LIF (and not FGF2). At embryonic day 8 in the mouse, FGF2 dependent neural stem cell can be isolated from the developing neural plate.

Please replace the Abstract on page 77 with the following:

ABSTRACT OF THE DISCLOSURE

Described are a novel cell type in the neural lineage, and method of producing the same based on the degree of neural commitment and growth factor responsiveness in vitro and the potential to give rise to neural and non-neural progeny in vivo. The novel vell type of neural lineage and cells derived therefrom have a number of applications including applications regarding tissue engineering, transplantation and gene therapy [therap] and drug discovery. Also described are suggested uses of the method and cell type including isolating genes that positively and negatively regulate the transition from an ES cell to a neural cell and generally for studying ES cell models of mammalian neural development.